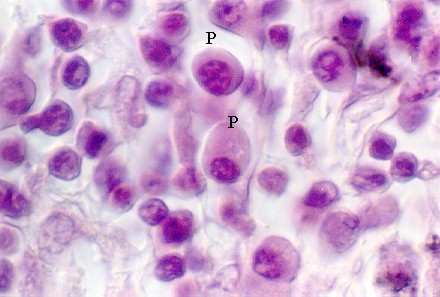
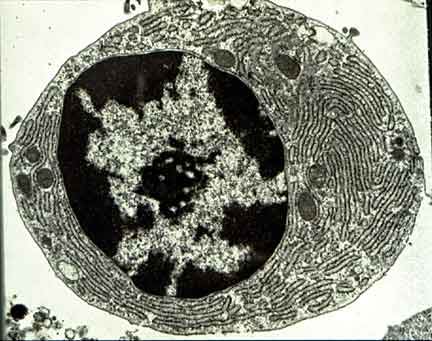
**LABORATORY DEMONSTRATION FOR CONNECTIVE TISSUE**

# PLASMA CELLS

### HIGH POWER

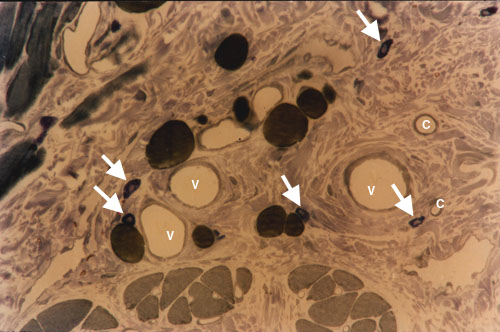
These cells (**arrows**) have a large amount of cytoplasm with the nucleus eccentrically located. The chromatin pattern has a "cartwheel" appearance. These cells are found only in connective tissue. What is the origin of these cells and what do they do? 630X

****

# MAST CELLS

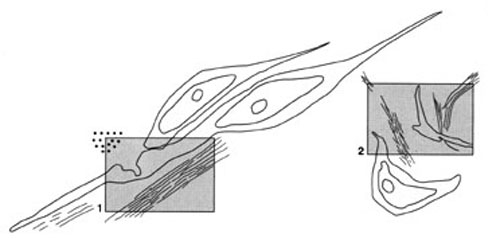
### HIGH POWER

Toluidine blue stains the granules in the mast cells (**arrows**) dark blue. The nucleus is visible as a light oval within the cell. Mast cells are typically associated with capillaries (**C**)and venules(**V**).



# COLLAGEN AND ELASTIC FIBERS

### ELECTRON MICROGRAPH

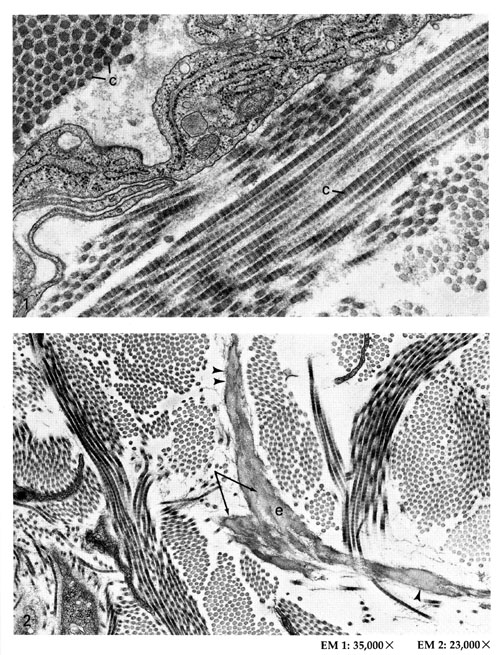


**Collagen**, visible in the light microscope with a diameter of 0.5 to 20um, is made up of smaller units, **fibrils**. In electron micrographs of loose and dense connective tissue, collagen fibrils (c, micrograph 1), with a diameter of 20 to 100 nm, are visible in cross section. In longitudinal section striations with a periodicity of 67nm are apparent. These are created by the staggered arrangement of the individual tropocollagen molecules separated end to end by a gap of 35nm. Additional narrow bands within this pattern represent stain binding to polar residues of tropocollagen lined up in register.

Like collagen, **elastic fibers** are synthesized by fibroblasts and are ubiquitous. In contrast to collagen, these relatively thin (0.2-1um) branching fibers are not composed of smaller striated fibrils. One elastic fiber viewed under the light microscope is seen as a single structure in routine electron micrographs. The arrows in micrograph 2 identify an elastic fiber that appears to be branching. Each fiber consits of (1) **elastin** (e), a unique protein with primarily hydrophobic, nonpolar amino acids, which does not stain and appears as an amorphous strip, and (2) stained 10-nm **microfibrillar proteins** (arrowheads) containing hydrophilic residues that form a sheet around the elastin.

The resilient character of elastin, which is particularly important in the lung, aorta, and skin, is due in part to the intermolecular cross-links that form extracellularly between the tropoelastin molecules. A defect in lysyloxidase, an enzxyme necessary for both the cross-linking of tropocollagen and tropoelastin, results in hyperextensible skin and joints, one type of Ehlers-Danlos syndrome.

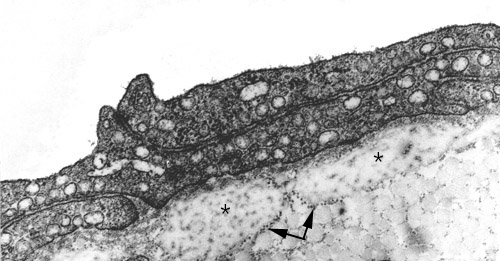
Extracellular fibers attach to cells either directly via membrane receptors (as with elastin) or indirectly via other molecules such as laminin (in case of collagen type IV) and fibronectin (with collagen types I-III). This association can influence gene expression and functions in many diverse ways. The elastic fiber receptor holds recently synthesized tropoelastin in proper orientation for cross-linking and final fiber formation. Cross & Mercer - Cell and Tissue Ultrastructure p72, 1993.



# ELASTIC FIBER

### ELECTRON MICROGRAPH

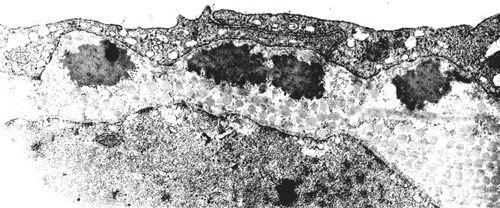
Cross sections of two elastic fibers lying subjacent to ehdothelial cells of a blood vessel. The elastic fiber consists of an amorphous material (electron lucent \*) and dark staining elastic microfibrils (**arrows**) at the periphery of the fiber. Urynal acetate, lead citrate stain. Crissman 56,000X



# ELASTIC FIBER

### ELECTRON MICROGRAPH

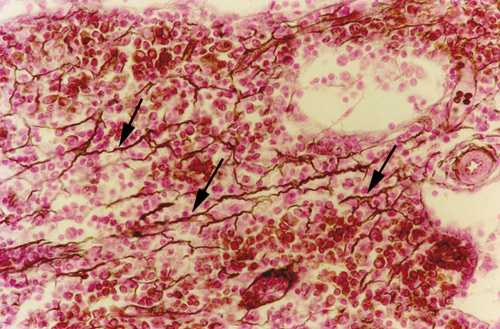
Cross section of four elastic fibers lying subjacent to endothelial cells of a blood vessel. These fibers are darkly stained with Verhoeff's iron hematoxylin which is specific for elastic fibers. Note the numerous collagen fibers in cross section surrounding the elastic fibers. Crissman 56,000X



# RETICULAR FIBERS

### HIGH POWER

These black, argyophilic, extracellular fibers (**arrows**) form thin delicate networks in reticular tissue of lymphatic organs and bone marrow. These fibers typically intersect at right angles. 400X



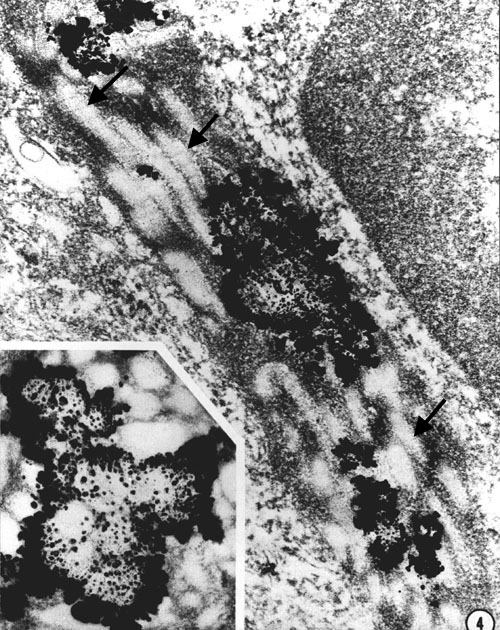
# RETICULAR FIBERS

### ELECTRON MICROGRAPHS

UPPER. Electron micrographs demonstrating the lightly staining collagenous fibrils embedded in a dark matrix. Reticular fibers (**circles**) are usually encircled by slender cytoplastic arms of reticular cells. 15,000X

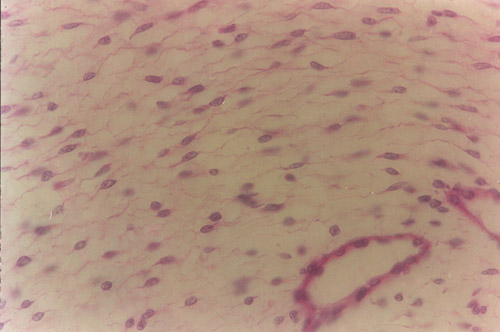
LOWER. Tangential section of a single reticular fiber. Faintly cross banded collagen fibrils (collagen type III) surrounded by a darker fuzzy coat of polysaccharides make up the reticular fiber. The intense black is the silver stain localizing in the sugar coat. 65,000X: inset 105,000X Snodgrass, 1978





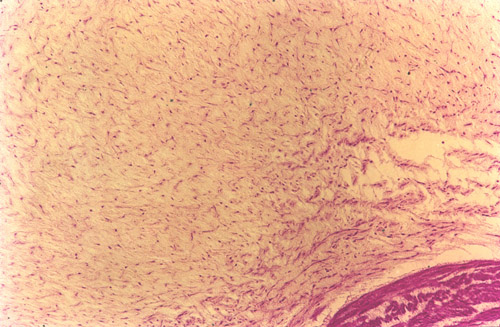
# MESENCHYMAL TISSUE

This embryonic tissue is characterized by relatively numerous cells, large amounts of amorphous ground substance, and very few, fine extracellular fibers. What can these mesenchymal cells differentiate into? 400x



# MUCOID CONNECTIVE TISSUE

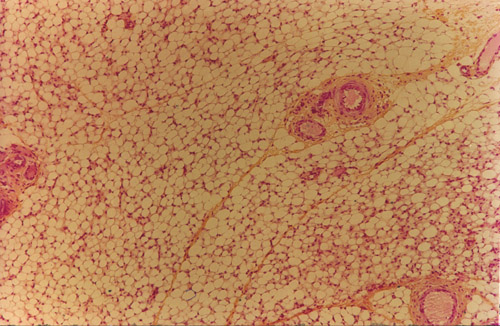
It is characterized by scattered fibroblasts, few extracellular fibers, and lots of amorphous ground substance filled with proteoglycans which have condensed to give the appearance of fibers. It is found in the umbilical cord as "Wharton's jelly". 100X



# ADIPOSE TISSUE

### LOW POWER

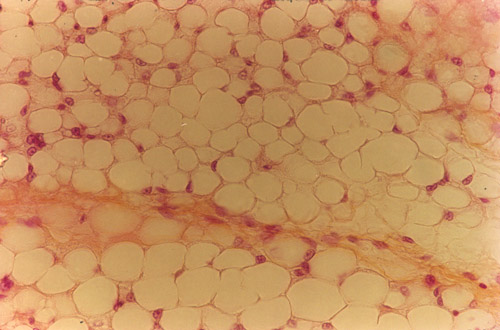
This tissue is characterized as a lacey network of cells with delicate septa (that contain blood vessels and nerves) of areolar connective tissue running through it. What are normal locations of this tissue in the body? 100X



# ADIPOSE TISSUE

### HIGH POWER

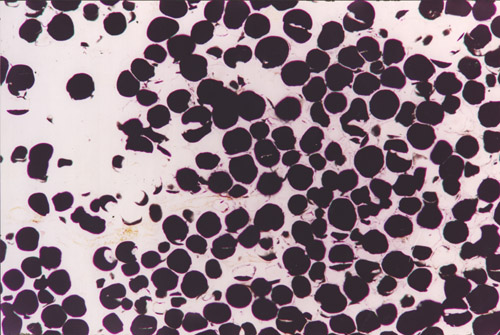
Adipose cells, fixed and processed with normal LM processing has the lipid extracted, appear as large thin rings of cytoplasm with the flattened nucleus off to one side. This is the typical "Signet Ring" appearance of an adipocyte. 400X



# ADIPOSE TISSUE

### HIGH POWER

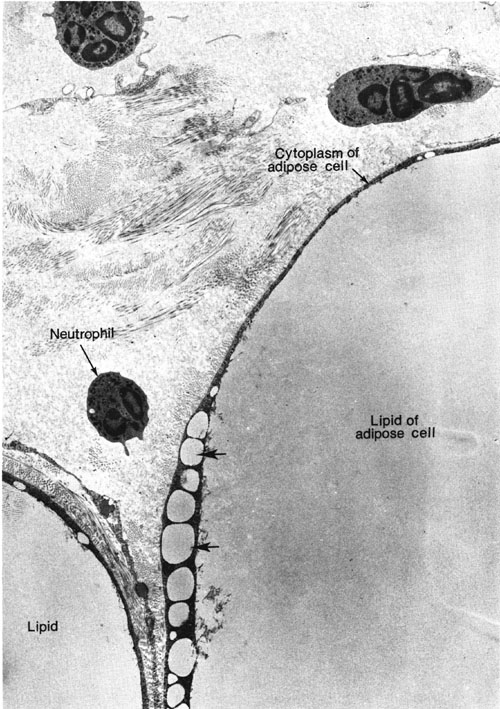
The large dark staining spots are adipocytes fixed with osmium tetroxide to preserve the lipids. Is this "white" or "brown" fat? 400X



# ADIPOSE CELL

### ELECTRON MICROGRAPH

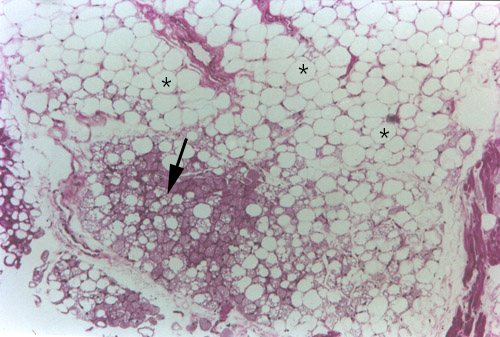
Parts of two white adipose cells and surrounding connective tissue. The cytoplasm of the cell has been displaced to a thin line around a single globule of fat. The nucleus is also displaced but not seen here. The newly synthesized triglycerides are first seen as small droplets (liposomes- **arrows**) in the cytoplasm before they coalesce with the large central lipid droplet. Fawcett -The Cell, 1981.



# WHITE AND BROWN FAT

### HIGH POWER

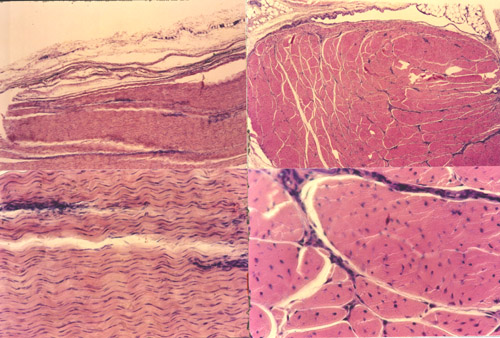
White (unilocular) fat cells (**\***) are characterized by the large round, empty spaces surrounded by a thin ring of cytoplasm. The brown (multilocular) fat cells (**arrow**) are characterized by numerous small extracted lipid droplets scattered within the darker cytoplasm.



# DENSE REGULAR CONNECTIVE TISSUE

### LONGITUDINAL & CROSS SECTIONS AT LOW & HIGH POWER

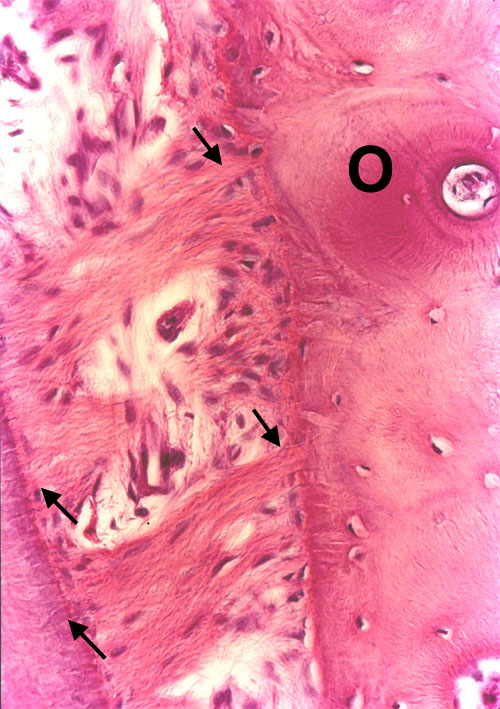
This connective tissue is characterized by numerous collagen fibers packed in the extracellular matrix as seen in longitudinal (left) and cross sections (right). The fibers are parallel and oriented in the same direction - along the axis of stress. Very little ground substance is seen. At higher magnification in longitudinal section (bottom right), the fibrocyte nuclei appear flattened between the packed collagen fibers. In cross section, the nuclei appear scattered throughout the bundles and are more stellate in appearance. How do you distinguish this tissue from skeletal muscle in cross section?



# SHARPEY'S FIBERS

### HIGH POWER

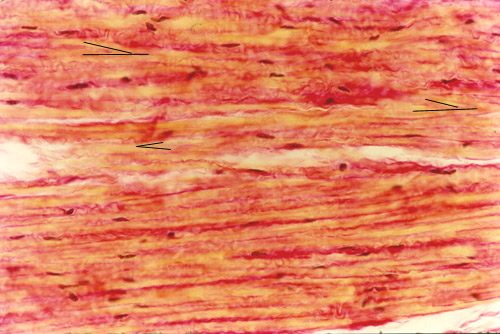
Bundles of type I collagen fibers form the periodontal ligament that holds the tooth in place. The Sharpey's fiber (arrows) are embedded in bone tissue of the mandible (note osteon) as well as in the cementum of the tooth. 400X



# ELASTIC TISSUE, LONGITUDINAL SECTION

### HIGH POWER

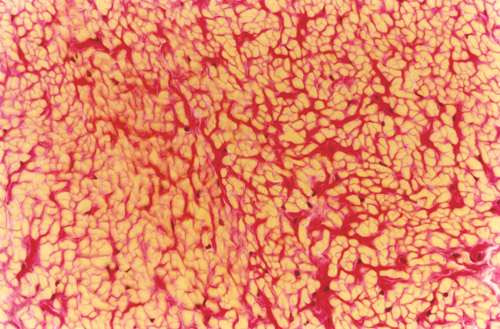
The yellow staining elastic fibers are straight and "Y" branching. Broken fibers have a chacteristic "S-shaped" squiggle. There is not much amorphous staining ground substance. 400X



# ELASTIC TISSUE, CROSS SECTION

### HIGH POWER

This dense connective tissue is characterized by tightly packed elastic fibers (yellow) surrounded by a thin layer of areolar connective tissue (red). Dark staining fibroblasts are wedged in between the fibers. 400X



# BASEMENT MEMBRANE

### ELECTRON MICROGRAPH

The basement membrane is composed of 3 regions at the ultrastructural level. These are the lamina lucida (light area immediately adjacent to the cell membrane); the lamina densa (which is labelled as basal lamina); and the lamina fibroreticularis. Fawcett- The Cell, 1981

